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INSECTICIDE RESIDUES IN MEAT AND MILK

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The chlorinated hydrocarbon and some of the organic phosphorus insecticides are fat-soluble and when sprayed on cattle or sheep may be absorbed through the skin and stored in the fatty tissues. They also may be stored in the fat when present as contaminants of feed, and they may be excreted in the milk of dairy cows exposed to them in sprays or feed. Therefore, before an insecticide can be recommended for use on livestock or for the control of insects on pasture and forage crops, studies must be made to determine whether or not the dosages used will lead to meat and milk contamination, and if residues are produced how long they will persist.

Such studies have been conducted at the Kerrville, Tex., laboratory during the last six years. This work has been carried out through the cooperation of entomologists, veterinarians, and chemists of the Agricultural Research Service.[1]

Methods of Analysis

The fat samples were supplied by the veterinarians, who used the biopsy technique described by Radeleff (15). Samples were placed in paper cartons and refrigerated at 0°-5° C. until dehydrated, which required about 6 days. The dehydrated fat was weighed, cut into small pieces, placed in a Waring Blendor with 125 ml. of the proper solvent, and blended for 2 minutes. Twenty grams of anhydrous sodium sulfate was added, and the mixture was blended for 1 minute longer. Then 1 gram of Filter Cel was added, well mixed, and the extract filtered. The Blendor and filter were washed with enough solvent to make a total volume of 200 ml. The filtered extracts were then variously treated for fat removal and analysis.

1/ In addition to the author, the following individuals participated in these studies: R. D. Radeleff, W. J. Nickerson, and G. T. Woodard of the Animal Disease and Parasite Research Branch, and R. C. Bushland, R. W. Wells, H. D. Mann, H. F. Beckman, M. C. Ivey, and J. W. Bowers of the Entomology Research Branch

The methods of extracting the insecticides from milk were different from those used for the extraction from body fat. However, the butterfat was always removed with the insecticide, and the same methods of fat removal and analysis were used in both determinations.

For DDT and TDE analyses, chloroform was the solvent used in the extraction. In early experiments the removal of fat and determination of the insecticides were made by the method described by Schechter et al. (17). In later experiments the method was modified so that larger fat samples could be used.

For methoxychlor in fat, n-hexane was used in the extraction. The fat was removed and the methoxychlor determined by the method of Claborn and Beckman (3). Methoxychlor in milk was determined by the method described by Prickett et al. (14), since this work was done before the development of the method by Claborn and Beckman (3).

For lindane analysis, n-hexane was used for the extraction solvent and lindane was determined by the spectrophotometric method described by Davidow and Woodard (8).

For toxaphene, chlordane, gamma chlordane, heptachlor, dieldrin, aldrin, and endrin analyses, benzene was the solvent used for the fat extraction, and the samples were analyzed for organically bound chlorine. The method described by Carter (1) was used in the early experiments; later (where the insecticides are recorded to 0.1 p.p.m.) other more sensitive methods were used. Strobane, heptachlor, chlordane, and gamma chlordane were extracted from the fat with nitromethane, the organic chlorine was reduced with sodium, and the chlorides were titrated amperometrically with silver nitrate. The method is sensitive to $\pm$ 0.5 p.p.m. on a 25-gram sample of fat.

In our most recent experiments a combustion method developed by C. L. Dunn (unpublished) of Hercules Powder Company has been used for determining total chlorine. A sample of fat is burned and the chlorides titrated amperometrically with silver nitrate. This combustion method used in conjunction with a washing procedure to remove inorganic halides from butterfat and beef fat has proved to be the most satisfactory method we have tried. The complete removal of water-soluble materials greatly reduces the blank and the variability in control samples.

For determination of the alkali-stable insecticides--aldrin, dieldrin, and endrin--the fat was saponified with alcoholic potassium hydroxide, the resulting soap solution extracted with petroleum ether (Skellysolve B), and the extract concentrated, burned, and chlorides titrated with silver nitrate (13). In the later experiments the combustion step was replaced with sodium reduction.

Malathion was determined by a chlorimetric method described by Norris et al. (12) of American Cyanamid Company and modified for milk

and fat analyses by M. V. Norris and L. P. Fuller (private communication). The method is sensitive to ± 0.5 p.p.m. on a 20-gram sample of fat and to ± 0.02 p.p.m. on a 1000-gram sample of milk.

Perthane was determined by a spectrophotometric method developed by A. R. Weiss (unpublished) of Rohm & Haas Company.

Dilan was determined by a colorimetric method developed by Jones and Riddick (10, 11) and modified by R. H. Cundiff (unpublished) of Commercial Solvents Corporation.

Storage in Fat of Beef Cattle Receiving Spray Treatments

Single Treatments (4)

Groups of four 6-month-old calves (2 steers and 2 heifers) were sprayed with a xylene emulsion of DDT, TDE, methoxychlor, or lindane. Fat samples were taken from all the animals before treatment and 2, 6, and 10 weeks after treatment, and other samples were taken when necessary to determine the duration of fat contamination. The analytical results are shown in table 1.

TDE was stored in slightly less amount than DDT. The storage of methoxychlor was only about one-fourth as great as that of DDT, and lindane was not detected in the fat. The methoxychlor residues were eliminated in 10 weeks, but DDT and TDE were detected in significant amounts 27 weeks after treatment.

Multiple Treatments

DDT, TDE, Methoxychlor, and Lindane (7).--Four groups of 6 calves (3 steers and 3 heifers) were sprayed with one of these insecticides. The spray concentrations and formulations were the same as were used in the single treatments, but the treatments were repeated six times at intervals of 3 weeks. Samples of fat were taken from all 6 calves of each group before the first treatment, and from 3 calves of each group 3 weeks after the first, second, fourth, and sixth treatments and then at 12, 24, and 36 weeks after the last spraying. The results of analyses of fat samples are reported in table 2.

The storage of DDT and TDE following the second spray treatment was almost twice as great as the storage after the first treatment, but further spray treatments caused no significant increases in the insecticide storage. The values for methoxychlor were variable, but the highest value was obtained after the sixth spraying.

The amounts of DDT and TDE in the fat 3 weeks after the first spraying in this experiment were greater than were found 2 weeks after spraying in the single-spray experiment. These results may be explained

by the fact that in the single experiment the cattle were sprayed in July when the hair was short and in this experiment the first spray was applied in late December when the hair was long and could hold more of the insecticides.

Excessive Treatments with DDT.--The results with DDT indicated that the storage of the insecticide in the fat reached a maximum after six applications. An experiment was then run to determine the residues that would result from spray treatments over an extended period of time.

Fat samples were taken from six 6-months-old calves and analyzed for DDT. Before treatment the DDT content ranged from 5 to 75 p.p.m. and averaged 45 p.p.m. These calves were sprayed with 0.5-percent DDT emulsion five times at 3-week intervals and then after 5 days were given 28 treatments at 2-week intervals. The DDT content of the fat samples taken 2 weeks after the last treatment ranged from 76 to 89 p.p.m. and averaged 84 p.p.m. The one animal that had 75 p.p.m. at the start had only 81 p.p.m. after 33 additional sprayings.

Another group of 6 calves, fat samples from which were negative for DDT before treatment, were sprayed 28 times at 2-week intervals. Fat samples taken from 5 of the animals 2 weeks after the last treatment contained from 80 to 103 p.p.m. of DDT, and the average was 88 p.p.m., 4 p.p.m. higher than for the first group.

Cows and Calves Sprayed with DDT.--Eight Hereford cows with young calves were used for this experiment. All the cows and 4 of the calves were sprayed five times at 28-day intervals with 0.5-percent DDT, half these animals with an emulsion and the other half with a suspension. The other 4 calves were not sprayed. Each calf lived upon the milk supplied by its mother. All the animals were slaughtered 28 days after the last spraying.

An average of 52 p.p.m. of DDT was found in the fat from the calves that were getting DDT both by absorption and ingestion and 25 p.p.m. from those that were getting DDT only from the milk. More DDT was stored in the fat of the calves that were not sprayed than in the fat of their mothers, which averaged only 15 p.p.m.

Dieldrin.--Three heifers were sprayed four times at 3-week intervals with 0.05-percent dieldrin. Analyses of fat samples taken after each spraying and 11 and 28 weeks after the last spraying are given in table 2.

Heptachlor, Chlordane, and Gamma Chlordane.--This experiment was conducted in cooperation with the Velsicol Corporation. Four calves were sprayed six times at 2-week intervals with a xylene emulsion containing 0.5 percent of gamma chlordane, and 4 other calves were sprayed in the same manner with an emulsion of heptachlor. A third group of

4 calves were used for controls. Fat samples were taken by biopsy for chemical analysis. Samples were taken from all animals before spraying; from 2 calves from each group 2 weeks after each of the first five sprayings; and from all animals 2, 8, and 16 weeks after the sixth spraying.

Four calves in good condition were sprayed 12 times at 2-week intervals with 0.5-percent emulsion of chlordane. Four other calves in very poor condition were given the same treatment. Fat samples were taken before treatment and 2, 8, and 16 weeks after the last spraying.

The fat samples were analyzed and the results are reported in table 2.

Toxaphene and Strobane.--In a cooperative experiment with the Humble Oil and Refining Company, fat samples were taken from cattle sprayed 12 times at 2-week intervals with toxaphene applied at 0.5-percent concentration. Six animals were sprayed with a xylene emulsion formulated at Kerrville and 6 with an emulsion prepared from a concentrate furnished by the company. In each group fat samples were taken from all animals before the treatments--from 3 animals 2 weeks after each of the first six sprayings, from 2 animals 2 weeks after each of the last six sprayings, from 1 animal 4 weeks after the last spraying, and from two 2 weeks later. The analytical results are shown in table 3.

In a similar experiment in which the B. F. Goodrich Chemical Company cooperated, cattle were sprayed 12 times at 2-week intervals with 0.5-percent Strobane, 6 with an emulsion and 6 with a suspension. Fat samples were taken at indicated intervals after spray treatments. The analytical results are shown in table 4.

Six cattle were sprayed six times at 2-week intervals with 2-percent Strobane. Fat samples were taken before the first spray treatment and 6, 10, and 14 weeks after the last spraying. The fat analyses are reported in table 2.

Malathion.--In cooperation with the American Cyanamid Company, 8 calves were sprayed 16 times at weekly intervals with 0.5-percent malathion, 4 with an emulsion and 4 with a suspension. Fat samples were taken before the first spraying and 2 weeks after the last spraying. No malathion was detected in any of the samples.

Storage in Fat of Beef Cattle and Sheep Following Ingestion of Contaminated Feed

The great variation in the residues left on forage due to methods of application, differences in growth rate of individual crops, and weather conditions made it difficult to determine what concentrations of insecticides would normally be encountered on forage crops. Since the Cereal

and Forage Insects Section of this Branch gathered crop-residue data, their results were considered when these experiments were planned. In the first tests the insecticides were added to feed in dosages likely to exceed field contamination. If the storage seemed excessive, further tests were made at progressively lower dosages until the minimum field residues were studied.

The feed consisted of ground corn and oats, cottonseed meal, and chopped alfalfa hay. The hay was incorporated with the feed concentrate and fed as one mixture. The insecticides were dissolved in acetone in such concentrations that 1 ml. of solution was sufficient to contaminate 1 pound of feed to the desired degree. Each feeding was weighed and the insecticide added at the time of weighing. The test animals were maintained under the supervision of the veterinarians, who were responsible for their feeding.

The cattle were fed in individual stalls twice daily and were given all the feed they would eat. The sheep were grouped as ewes or wethers and fed in groups of 2 or 3; otherwise the feeding was the same as for the cattle. The sheep were kept in small pens throughout the test period, but the cattle were allowed freedom in an exercise lot when not being fed.

The first studies covered a feeding period of only 4 weeks. Later the time was extended to 8, 14, and then to 16 weeks, which is the maximum time that cattle and sheep would be kept on feed when being finished for slaughter. Samples were taken before the feeding period started, at 2- or 4-week intervals during feeding, and at indicated intervals after the feeding of the insecticides ceased. The analyses of the fat samples are shown in table 5.

Methoxychlor is the only insecticide studied that did not cause some storage in the fat. Of all the insecticides we have studied the order of their storage in the fat is as follows: aldrin $>$ dieldrin $>$ BHC $>$ DDT $>$ chlordane $>$ lindane $>$ endrin $>$ heptachlor $>$ toxaphene $>$ methoxychlor.

Contamination of Milk

Rate of Excretion of Different Insecticides from Spray Treatment

Since Howell *et al.* (9) discovered that DDT was excreted in the milk of cows sprayed with this insecticide, many studies have been made to determine the rate of excretion and the duration of the contamination. Less work has been done on the other chlorinated hydrocarbon insecticides because they have not been used so extensively on livestock or because methods of analyses of comparable sensitivity were not available.

During the last 7 years data have been accumulated for a comparison of the typical rates and duration of excretion after treatments with sprays containing 0.5 percent of DDT, methoxychlor, dieldrin, Dilan, Perthane, malathion, toxaphene, and Strobane. A 0.5-percent concentration is practical for horn fly control with DDT, methoxychlor, Dilan, Perthane,

malathion, toxaphene, and Strobane, but dieldrin should be used at a lower concentration. It was used at 0.5 percent in these tests in order that rates of excretion could be compared.

The cows used in this experiment were good-grade Jerseys in full lactation. They were sprayed all over, 2 quarts being used for each cow. They were milked by machine, and the samples were taken from the full yield. Great care was taken to avoid mechanical contamination of the milk. Since all the insecticides used are fat-soluble and are known to be present only in the butterfat of milk, the butterfat of each sample was determined, and the results were adjusted to a uniform butterfat content of 4 percent. The milk samples were taken before spraying and at various intervals thereafter.

Control cows were used in all experiments. These cows received the same feed, and milk samples were taken from them at the same time they were taken from the treated cows. In all the experiments the samples from the control cows were negative.

DDT.--Although the excretion of DDT in milk following treatment of commercial dairies was studied in 1949 (Claborn et al. (5) this work was repeated in order that the treatment would be uniform for all materials. Three cows were sprayed with a 0.5-percent suspension. The results are given in table 6.

Dieldrin.--One cow was sprayed with a 0.5-percent dieldrin emulsion. The results, given in table 7, agree very closely with bioassay data obtained by Y. P. Sun of Julius Hyman and Company on milk samples from two other cows similarly treated.

Methoxychlor.--Carter et al. (2) found that the presence of methoxychlor in milk following spray treatments could not be definitely shown by the organic-chlorine method, which at the time of their study was the only method available. The development of a sensitive colorimetric method by Prickett et al. (14) made it possible to make more precise determinations.

One cow was sprayed with a 0.5-percent methoxychlor emulsion and another cow with the same concentration of a suspension. The excretion of methoxychlor had reached zero at the end of 21 days, and the test was repeated on the same cows. Results of the analyses are given in table 7. The four samples collected from these 2 cows averaged 0.42 p.p.m. 2 days after spraying, and all were practically zero after 14 days. The average of all samples analyzed over the 21-day period was 0.19 p.p.m.

Following the studies with individually treated animals experiments were conducted on a herd basis. There had previously been some evidence with other insecticides that lower residues result when groups of animals are sprayed. Two herds of dairy cattle were sprayed at Kerrville, Tex., with a 0.5-percent methoxychlor suspension made from a wettable powder.

Application was at a rate of 2 quarts per animal. Pooled milk samples from one herd showed 0.18, 0.13, 0.11, and 0.05 p.p.m. of methoxychlor to be present 1, 2, 3, and 5 days after spraying. On the 7th, 14th, and 21st day, small amounts, less than 0.05 p.p.m., were recovered in the milk. These figures were adjusted to a uniform 4 percent butterfat content. Similar data were obtained from another sprayed herd. Results of these studies will be published elsewhere in more detail in the near future.

Dilan.--Two cows were sprayed twice 14 days apart with a 0.5-percent suspension of Dilan. Milk samples were collected at various intervals during the 14 days after the first spraying and 21 days after the second spraying. Results of the analyses are given in table 8.

Malathion.--Four cows were sprayed twice, 1 week apart, with malathion, 2 with emulsions and 2 with suspensions each at 0.5- and 1.0-percent concentrations. Samples were taken at various intervals and freeze-dried, most of them immediately after they were taken, and no samples were allowed to stand in the refrigerator more than 24 hours. The cows excreted malathion ranging from 0.08 to 0.36 p.p.m. in all samples of milk taken 5 hours after the spraying. Traces were found in samples taken after 1 day, but samples taken after 3 and 7 days were free of contamination. The higher concentrations caused heavier residues than the lower concentrations and the suspensions caused heavier residues than the same concentrations applied as emulsions.

Perthane.--Two cows were sprayed twice, 3 weeks apart, with an emulsion and 2 with a suspension of Perthane, and 2 cows were sprayed twice daily for 21 days with 1 ounce of an oil solution. All the sprays contained 0.5 percent of Perthane. All the milk samples were analyzed by chemists of Rohm & Haas Company; and duplicate samples from 2 of the cows were analyzed at Kerrville. The results of the analyses are shown in table 10. These results are confusing since the higher values appear at random instead of at some definite time after spraying. Such variations clearly indicate the unreliability of the method. Since the maximum value found was lower than the maximum value found for methoxychlor, it would appear that Perthane sprays cause less contamination of milk than methoxychlor sprays of the same concentration.

Toxaphene and Strobane.--Six cows were sprayed with toxaphene and 6 with Strobane, and 2 cows were used for controls. Of the treated cows, 4 were sprayed twice three weeks apart, 2 with an emulsion and 2 with a suspension, and 2 were sprayed twice daily for 21 days with a 2-percent oil spray. The analyses of the samples from the emulsion- and suspension-sprayed cows are shown in table 11, and from the oil-sprayed

cows in table 9. The maximum contamination occurred 1 and 2 days after spraying. There was no significant difference in contamination caused by the suspensions and emulsions, or by the two insecticides.

Milk from Commercial Dairies in which Barns and Cows Were Sprayed with DDT or TDE

During the summer of 1948 DDT and TDE were tested for fly conrol at 7 commercial dairies (Claborn et al. 5). The insecticides were used in 5-percent emulsions for spraying barns and 0.5 percent for spraying the cattle. The barns were sprayed as often as needed for house fly control, and the cattle whenever horn flies became annoying. All the test herds excreted detectable amounts of insecticide in their milk throughout the fly season (May to September). The average contamination was 0.21 p.p.m. for the four herds treated with DDT and 0.25 p.p.m. for the three treated with TDE.

Source of Contamination of Milk from Sprays Applied to Dairy Barns

A study was made to determine the source of milk contamination when dairy barns were sprayed with 2.5-percent DDT. Three experiments showed that (1) the insecticide was actually secreted in the milk and did not get into it from mishandling of the milk or equipment, (2) no contamination resulted from inhalation of the insecticide by the cows, and (3) the insecticides excreted in the milk came from residues left on the feed troughs. Contamination did not occur when feed troughs were completely covered during the spraying or were washed afterwards.

Summary

Studies were conducted at Kerrville, Tex., to determine whether insecticides used on livestock or on pasture and forage crops will contaminate the meat or milk, and if residues are produced how long they will persist.

Single spray treatments of DDT, TDE, and methoxychlor applied at 0.5-percent concentration to beef cattle caused storage of the insecticide in the fat. Lindane at a concentration of 0.03-percent could not be detected.

Multiple spray treatments with lindane and methoxychlor at 3-week intervals caused no greater storage in the fat than a single treatment. Multiple treatments with other chlorinated hydrocarbon insecticides--chlordane, gamma chlordane, DDT, dieldrin, heptachlor, methoxychlor, TDE, Strobane, and toxaphene--at 2- or 3-week intervals resulted in slight to moderate increases in the amount of storage. A 0.5-percent malathion spray applied at weekly intervals caused no storage in the fat.

When insecticides were fed to beef cattle and sheep as a contaminant of their feed at dosages likely to occur as residues on forage crops, all except methoxychlor were stored in the fat. The order of their storage was as follows: aldrin > dieldrin > BHC > DDT > chlordane > lindane > endrin > heptachlor > toxaphene.

All the chlorinated hydrocarbon insecticides and malathion were excreted in the milk of dairy cows following the spray treatments. Dieldrin caused the greatest contamination, followed in order by DDT, TDE, Dilan, toxaphene, Strobane, methoxychlor, Perthane, and malathion.

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Table 1.--Parts per million of insecticide in the fat of cattle at different times after a single spray treatment.

Insecticide	Animal	2 weeks	6 weeks	10 weeks	16 weeks	22 weeks	27 weeks
DDT 0.5%	Steer	5.2	5.1	2.1	1.7	2.2	1.1
		8.9	5.7	5.5	1.9	3.2	2.0
	Heifer	13.8	9.9	5.2	2.2	3.0	1.6
		16.9	11.9	8.4	3.4	3.1	2.1
	Av.	11.2	8.1	5.3	2.3	2.8	1.7
TDE 0.5%	Steer	7.8	3.7	3.4	0.5	0.5	0.4
		12.3	4.2	3.2	-	.9	.4
	Heifer	8.4	4.1	3.5	1.0	.5	.4
		15.6	9.0	5.5	1.9	.9	.7
	Av.	11.0	5.2	3.9	1.1	.7	.5
Methoxychlor 0.5%	Steer	2.4	1.8	0			
		2.4	0.8	-			
	Heifer	2.8	1.8	-			
		3.8	2.4	0			
	Av.	2.8	1.7				
Lindane 0.03%			None in either steers or heifers				

Table 2.--Parts per million of insecticides in the fat of cattle after multiple spray treatments.

Insecticide	Spray interval (Weeks)	Animal	After indicated spraying[1]						After last spraying		
			1st	2nd	3rd	4th	5th	6th	12 weeks	24 weeks	36 weeks
DDT 0.5%	3	Steer	16.2	-	-	33.9	-	-	6.3	-	2.9
			15.7	-	-	37.6	-	-	9.1	-	1.8
			-	32.4	-	-	-	-	-	4.9	-
		Heifer	22.1	-	-	27.0	-	-	7.8	-	1.9
			-	29.5	-	-	-	32.2	-	4.8	-
			-	31.6	-	-	-	38.2	-	4.4	-
		Av.	18.0	31.2	-	32.8	-	35.2	7.7	4.7	2.2
TDE 0.5%	3	Steer	8.4	-	-	30.6	-	-	9.2	-	-
			23.0	-	-	37.7	-	-	9.5	-	0.8
			-	36.6	-	-	-	-	-	1.2	-
		Heifer	8.4	-	-	41.2	-	-	22.3	-	0.8
			-	25.0	-	-	-	28.4	-	1.4	-
			-	36.6	-	-	-	28.4	-	1.2	-
		Av.	13.2	32.7	-	36.5	-	28.4	13.6	1.3	0.8
Methoxychlor 0.5%	3	Heifer	1.6	-	-	0.8	-	-	0		
			1.8	-	-	.6	-	-	0		
			1.0	-	-	.9	-	-	0		
		Steer	-	0.8	-	-	-	1.6			
			-	1.4	-	-	-	2.1			
			-	2.2	-	-	-	3.4			
		Av.	1.5	1.5	-	0.8	-	2.4			
Lindane 0.05%	3	Steer	None after any spraying								

									11 weeks	28 weeks	
Dieldrin 0.05%	3	Heifer	8	10	21	27	-	-	-	6	
			7	-	15	23	-	-	-	6	
			6	9	14	22	-	-	17	6	
		Av.	7	10	16	24	-	-	17	6	
									8 weeks	16 weeks	
Heptachlor 0.5%	2	Steer	9.2	-	11.9	-	15.0	16.7	11.2	0	
			-	10.2	-	16.6	-	15.4	-	-	
		Heifer	13.3	-	16.0	-	20.4	19.2	14.9	2.2	
			-	17.1	-	21.6	-	26.1	20.5	3.8	
		Av.	11.2	13.6	13.9	19.1	17.7	19.3	15.5	2.0	
Chlordane 0.5%	2	Steer	-	-	-	-	-	13.6	5.7	1.5	
			-	-	-	-	-	34.4	11.9	7.6	
		Heifer	-	-	-	-	-	18.9	7.8	-	
			-	-	-	-	-	15.8	6.6	4.5	
		Av.	-	-	-	-	-	20.7	8.0	4.5	
	2	Steer2/	-	-	-	-	-	34.0	-	7.5	
			-	-	-	-	-	-	-	2.4	
		Heifer2/	-	-	-	-	-	22.0	-	1.7	
		Av.	-	-	-	-	-	28.0	-	3.9	
Gamma chlordane 0.5%	2	Steer	-	7.0	-	14.8	-	22.0	15.2	0	
			11.9	-	14.7	-	18.0	-	-	0	
		Heifer	-	13.0	-	19.5	-	26.8	16.1	0	
			5.5	-	16.9	-	18.4	23.2	-	1.1	
		Av.	8.7	10.0	15.8	17.1	18.2	24.0	15.6	-	
									6 weeks	10 weeks	14 weeks
Strobane 2%	2	Steer	-	-	-	-	-	32.4	9.1	3.4	3.3
			-	-	-	-	-	33.9	14.4	6.0	4.1
			-	-	-	-	-	20.4	4.6	1.2	0.8
		Heifer	-	-	-	-	-	32.2	10.2	5.1	3.9
			-	-	-	-	-	23.6	6.9	3.0	1.6
			-	-	-	-	-	-	6.9	4.0	1.9
		Av.	-	-	-	-	-	28.5	8.7	3.8	2.6

1/ Fat samples were taken at the end of the intervals between spraying.
2/ Cattle were in poor condition.

Table 3.--Parts per million of toxaphene in fat of calves sprayed with 0.5-percent toxaphene[1/]

Animal	2 weeks after indicated spraying[2/]										After 12th spraying	
	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	4 weeks	6 weeks
	Emulsion prepared in laboratory											
Steer	0	-	7	-	7	-	-	10	-	13	-	-
	-	11	-	0	-	-	-	-	-	9	-	-
	-	3	-	0	-	10	-	-	10	-	-	0
	2	-	-	-	-	-	-	-	-	-	-	-
	7	-	17	-	-	8	-	-	12	-	-	5
	-	2	-	4	-	-	7	-	-	-	5	-
Av.	3	5	12	1	7	9	7	10	11	11	5	2
	Emulsion from concentrate furnished by Humble Oil and Refining Company											
Steer	2	-	9	-	-	-	-	9	-	13	-	-
	-	3	-	4	-	-	12	-	-	20	-	-
	-	11	-	5	-	9	-	-	6	-	-	2
	0	-	13	-	7	-	-	-	-	-	-	-
	0	-	7	-	-	7	-	-	8	-	-	6
	-	-	-	8	-	-	-	-	-	-	4	-
Av.	1	7	10	6	7	8	12	9	7	16	4	4

1/ All samples taken from the control animals were negative or within the experimental error of the method, which is ± 4 p.p.m.

2/ None found in any sample after the first two sprayings.

Table 4.--Parts per million of Strobane in fat of cattle sprayed with 0.5-percent Strobane emulsion or suspension

Animal	2 weeks after indicated spraying							6 weeks after last spraying
	1st	2nd	4th	6th	8th	10th	12th	
				Emulsion				
Steer	-	-	-	-	3.7	-	6.9	-
	-	-	7.2	-	4.4	-	6.5	-
	-	3.8	-	7.3	-	-	6.3	-
Heifer	-	6.4	-	8.8	-	6.9	4.8	1.4
	1.8	-	-	-	-	-	5.9	2.6
	3.8	-	-	-	-	-	9.4	4.3
Av.	2.8	5.1	7.2	8.0	4.0	6.9	6.6	2.8
				Suspension				
Steer	-	-	7.8	-	5.1	-	4.7	-
	-	-	6.4	-	6.8	-	6.5	-
	-	6.1	-	6.9	-	9.6	7.5	3.1
Heifer	-	5.6	-	9.5	-	5.5	5.0	-
	3.8	-	-	-	-	-	7.1	
	1.9	-	-	-	-	-	5.0	2.0
Av.	2.8	5.8	7.1	8.2	6.0	7.5	5.9	2.6

Table 5.--Parts per million of insecticides stored in the fat of cattle and sheep that had known amounts added to their diet

Insecticide	Dosage (p.p.m.)	Animal	After feeding 4 weeks	After feeding 8 weeks	After feeding 12 weeks	After feeding 16 weeks	After feeding ceased 4 weeks	After feeding ceased 8 weeks	After feeding ceased 20 weeks	After feeding ceased 32 weeks	After feeding ceased 36 weeks
Aldrin	25	Steer	58	79	-	-	46	36	19	9	-
			41	77	-	-	56	36	21	7	-
		Av.	50	78	-	-	51	36	20	8	-
		Wether	60	77	-	-	-	46	25	31	33
		Ewe	60	79	-	-	67	30	25	14	11
		Av.	60	78	-	-	67	38	25	23	22
	10	Ewe	-	47	51	57					
			-	52	52	52					
			38	62	36	47					
		Wether	-	45	48	57					
			-	46	-	59					
			-	64	50	59					
		Av.	38	53	49	55					
		Heifer	34	46	39	59					
			29	48	51	58					
			30	38	41	41					
			37	35	48	52					
			36	41	41	38					
		Av.	35	41	44	49					
	5	Ewe	11	17	14	13					
			8	11	21	20					
			7	8	-	15					

		Wether	4	14	14	9		
			5	11	20	18		
			6	7	-	19		
		Av.	7	11	17	17		
Tech. Aldrin 60%	5	Ewe	0	0	6	6		
			0	3	5	9		
			0	0	3	6		
		Wether	0	1	4	6		
			0	0	2	5		
			-	2	3	-		
		Av.	0	1	4	6		
		Heifer	0	2	4	5		
			0	-	8	-		
			2	3	11	3		
		Steer	0	6	-	7		
			0	4	10	8		
		Av.	0	4	8	6		
	2.5	Wether	4.6	-	5.6	5.3	-	2.6
			-	4.7	-	4.7	-	3.2
		Ewe	4.2	-	3.4	3.9	-	1.5
			-	4.7	-	4.2	-	3.5
		Av.	4.4	4.7	4.5	4.5	-	2.8
		Steer	3.9	-	-	5.9	-	1.8
			-	5.7	-	6.5	-	2.1
		Heifer	3.6	-	4.7	4.2	-	2.0
			-	3.5	-	4.3	-	0.9
		Av.	3.8	4.6	4.7	5.2	-	1.7

Table 5.--Continued

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
BHC	100	Steer	117	228	227	257	94	-	12		
			177	-	252	254	72	-	19		
			187	242	259	248	90	-	14		
		Heifer	137	213	220	228	95	-	24		
			122	209	194	264	71	-	14		
		Av.	159	223	230	250	84	-	17		
									16 weeks		
		Ewe	82	-	101	104	-	28	3		
			144	140	138	127	-	43	5		
			98	114	108	101	-	24	6		
		Wether	112	115	113	110	-	-	-		
			125	105	122	135	-	30	-		
			113	117	131	128	-	35	5		
		Av.	112	118	119	117	-	32	5		
									20 weeks		
Chlordane	25	Steer	9	18	-	-	16	5	0		
		Heifer	16	19	-	-	11	5	0		
		Av.	12	18	-	-	14	5	-		
		Wether	4	12	-	-	0				
		Ewe	11	13	-	-	0				
		Av.	8	12	-	-	0				

	10	Ewe	-	19	17	7				
			-	15	9	6				
		Wether	9	16	17	15				
			-	13	10	7				
			-	20	27	-				
		Av.	9	16	16	9				
		Heifer	8	12	9	-				
			11	15	10	9				
			12	12	10	10				
			-	15	11	17				
			13	11	10	9				
		Av.	11	13	10	11				
									16 weeks	24 weeks
DDT	25	Steer	-	29	-	38	11	-	-	4.5
			28	-	45	38	23	-	7.3	3.9
		Heifer	-	40	-	46	26	-	12.0	6.8
			15	-	39	37	16	-	13.7	7.6
		Av.	22	34	42	40	19	-	11.0	5.7
		Ewe	5	-	15	15	10	-	6.1	3.9
			-	10	-	13	7	-	6.1	3.2
		Wether	15	-	14	16	10	-	5.4	3.2
			-	15	-	17	8	-	4.5	2.7
		Av.	10	12	14	15	9	-	5.5	3.2
	10	Cattle (2)	6.8							
		Sheep (2)	3.1							
									20 weeks	32 weeks
Dieldrin	25	Steer	70	63	-	-	68	55	25	10
		Heifer	80	86	-	-	67	36	15	9
		Av.	75	74	-	-	68	46	20	10

Table 5.--Continued

Insecticide	Dosage (p.p.m.)	Animal	After feeding 4 weeks	After feeding 8 weeks	After feeding 12 weeks	After feeding 16 weeks	After feeding ceased 4 weeks	After feeding ceased 8 weeks	After feeding ceased 20 weeks	After feeding ceased 32 weeks	After feeding ceased 36 weeks
Dieldrin	25	Wether	39	70	-	-	58	37	32	33	31
		Ewe	48	68	-	-	43	45	32	-	10
		Av.	44	69	-	-	50	41	32	33	20
								16 weeks	24 weeks		
	10	Steer	-	29	-	48	29	19	9		
			18	-	37	45	22	16	8		
		Heifer	-	22	-	42	13	11	5		
			14	-	33	39	15	13	9		
		Av.	16	26	35	44	20	15	8		
		Wether	-	65	-	60	37	28	12		
			8	-	38	40	20	20	12		
			-	23	-	41	35	25	19		
			9	-	31	52	29	22	15		
		Av.	8	44	34	48	30	24	14		
	2.5	Wether	5.7	-	8.6	9.8	9.8				
			-	7.5	-	10.2	8.5				
		Ewe	7.7	-	11.0	10.4	9.7				
			-	4.6	-	11.5	7.0				
		Av.	6.7	6.0	9.8	10.5	8.8				
		Steer	6.9	-	9.4	11.3	5.2				
			-	13.4	-	14.8	7.3				
		Heifer	7.1	-	11.1	12.3	4.4				
			-	10.5	-	18.9	6.0				
		Av.	7.0	11.4	10.2	14.3	5.6				

	1	Steer	4.2	-	6.0	-	1.9
			-	5.3	-	5.5	2.4
		Av.	4.2	5.3	6.0	5.5	2.2
		Wether	-	3.9	-	-	2.1
		Ewe	1.8	-	2.0	2.6	2.4
		Av.	1.8	3.9	2.0	2.6	2.2
Endrin	5	Steer	1.4	-	2.5	1.9	
			-	2.2	-	-	
		Heifer	1.2	-	2.4	1.3	
			-	0.8	-	3.6	
		Av.	1.3	1.5	2.4	2.3	
		Wether	1.9	-	0.5	3.5	
			-	1.5	-	1.4	
		Ewe	1.1	-	1.2	1.7	
			-	2.4	-	-	
		Av.	1.5	2.0	0.8	2.2	
	2.5	Steer	0.9	-	0.4	1.6	
			-	2.8	-	1.0	
		Heifer	1.6	-	1.3	0	
			-	2.3	-	0.6	
		Av.	1.2	2.6	0.8	0.8	
		Wether	3.4	-	2.8	0.9	
			-	1.8	-	0.3	
			-	-	-	1.6	
			3.1	-	1.8	2.7	
		Av.	3.2	1.8	2.3	1.4	
Heptachlor	10	Steer	5	-	5	4	1
			3	-	2	2	0
		Heifer	-	-	-	-	2
			-	8	-	9	4
		Av.	4	8	3.5	7.5	1.7

Table 5.--Continued

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
Heptachlor	10	Ewe	-	8	-	6	6				
			6	-	-	10	-				
		Wether	-	9	-	9	6				
			5	-	8	7	3				
		Av.	5.5	8.5	8	8	5				
	2.5	Steer	1.5	-	0	0.5					
			-	0.9	-	-					
		Heifer	1.2	-	1.4	0					
			-	0.5	-	0					
		Av.	1.4	0.7	0.7	0.2					
		Wether	0.9	-	0	2.0					
			0	0	-	0.4					
		Ewe	1.2	-	0	3.4					
			-	0	-	2.6					
		Av.	1.0	0	0	2.1					
Methoxychlor	25	Steer	-	0	-	0					
			0	-	-	0					
		Heifer	-	0	-	0					
		Av.	0	0	-	0					
		Ewe	-	0	-	0					
			0	-	0	0					
		Wether	-	0	-	0					
			0	-	0	0					
		Av.	0	0	0	0					

Toxaphene	100	Ewe	29	30	-	30	19	0
			21	19	-	21	9	0
			19	18	25	19	11	0
		Wether	20	17	-	17	10	0
			25	19	-	19	15	0
			16	24	0	17	10	3
		Av.	22	21	25	20	12	0.5
		Steer	25	27	36	37	10	-
			-	45	43	52	29	9
			30	34	29	29	24	0
		Heifer	23	27	25	33	10	3
			26	35	33	39	15	0
		Av.	26	34	33	38	14	3
	25	Ewe	1	1	3	11		
			1	1	3	7		
			0	1	1	11		
		Wether	1	0	9	-		
			2	4	1	4		
			4	4	1	6		
		Av.	2	2	3	8		
		Heifer	2	4	11	16		
			3	4	7	12		
			1	9	9	16		
		Steer	4	4	11	8		
			1	1	12	9		
		Av.	2	4	10	12		
	10	Cattle (2)	4					
		Sheep (2)	4					

Table 5.--Continued

Insecticide	Dosage (p.p.m.)	Animal	After feeding			After feeding ceased			
			2 weeks	6 weeks	10 weeks	2 weeks	6 weeks	10 weeks	14 weeks
Lindane[2]	1	Heifer	No fat	1.0	1.3	1.6	-	0.9	-
		Steer	0.3	0.8	2.0	0.4	0.5	-	0.6
	10	Heifer	3.5	6.9	7.6	2.0	-	0.6	-
		Steer	No fat	6.7	8.3	4.2	4.9	-	-
	100	Heifer	59.0	76.0	86.0	40.0	-	3.7	1.0
		Steer	70.0	76.0	111.0	60.0	12.0	-	1.3

1/ Composite sample made up of equal amounts of six individual samples.

2/ These analyses were made by chemists of Hooker Electrochemical Co. The experiment has been described by Radeleff (16).

Table 6.--Parts per million of DDT in milk from three cows receiving one treatment with a 0.5-percent suspension.

Days	No. 1	No. 2	No. 3	Average
Before spraying	0.1	0.2	0.1	
After spraying				
2	3.8	3.0	1.8	2.8
7	1.1	2.0	1.2	1.4
14	0.5	0.5	1.0	0.7
21	.4	.4	0.9	.6

Table 7.--Parts per million of dieldrin and methoxychlor in milk from cows sprayed with 0.5-percent of these insecticides.

Days	Dieldrin emulsion	Methoxychlor	
		Emulsion	Suspension
Before spraying	0	0	0
After spraying			
1	2.9	0.70	0.25
2	5.5	.48	.44
3	7.0	.37	.33
4	3.8	--	--
5	--	.18	.19
7	1.7	.09	.16
10	--	.06	.05
14	1.3	.06	.05
22	0.4	0	0
Resprayed			
1	--	0.33	0.30
2	--	.40	.38
3	--	.30	.33
5	--	.18	.15
7	--	.09	.14
10	--	.06	.06
14	--	0	.06
21	--	0	0
Average	3.2	0.20	0.18

Table 8.--Parts per million of Dilan in milk from two cows sprayed with a 0.5-percent suspension.

Days	No. 1	No. 2	Average
Before spraying			
1	0	0	--
Same day	0.06	0.07	--
After spraying			
1	0.45	0.77	0.61
2	.70	.80	.75
3	.52	.83	.68
7	.92	.26	.59
10	.74	.33	.54
14	.62	.44	.53
Resprayed			
1	0.64	0.65	0.64
2	.96	1.26	1.11
3	.90	1.01	.96
7	1.07	.92	1.05
10	.52	.47	.50
14	.40	.30	.35
21	.20	.20	.20

Table 9.--Parts per million of Strobane and toxaphene in milk of cows sprayed twice daily with 1 ounce of 2-percent oil solutions of Strobane and toxaphene.

Days	Strobane		Toxaphene	
	No. 5	No. 6	No. 11	No. 12
After spraying started				
1	--	--	0.11	0.13
3	0.26	0.30	.32	.50
7	.27	.39	.30	.40
14	.30	.35	.20	.31
21	.30	.33	.25	.34
After spraying ceased				
7	0.08	0.06	0.09	0.09
14	0	.02	.10	.10
21	--	--	.07	.06

Table 10.--Parts per million of Perthane in milk from six cows sprayed with 0.5 percent of Perthane

Days after spraying	Emulsion				Suspension			Oil solution	
	No. 1		No. 2		No. 3		No. 4	No. 5	No. 6
	Kerrville	Rohm & Haas	Kerrville	Rohm & Haas	Kerrville	Rohm & Haas	Rohm & Haas	Rohm & Haas	Rohm & Haas
1	0.07	0.06	0.17	0.04	0.25	0.11	0.08	0	0
2	.10	.04	.18	.15	.05	.08	.22	0.09	0.10
3	.06	.12	.36	.22	.10	.07	.16	.08	.18
5	0	.13	.17	.28	.04	.12	.13	.10	.11
7	0	.12	.14	.07	.14	.04	.11	.05	.18
14	0	.17	.22	.02	.17	.04	.10	.13	.08
21	0	.01	.32	0	.12	.03	.03	.10	.14
Resprayed									
1	0	0.02	0.31	0.01	0.25	0.13	0.06	0.09	0.13
2	0	.11	.39	.03	.22	.08	.16	.11	.04
3	0	.11	.26	.04	.32	.03	.12	.03	.05
5	0.06	.03	.38	.04	.06	.10	.14	0	.04
7	.08	.04	.12	.04	0	.09	.02	.10	.07
14	0	.01	.12	.02	.05	.07	.05	.07	.12
21	0	0	0	.02	0	.05	0	0	.06
Average	0.03	0.07	0.22	0.07	0.13	0.07	0.10	0.07	0.09

Table 11.--Parts per million of Strobane and toxaphene in milk from cows sprayed twice at 3-week intervals with 0.5-percent of these insecticides

Days	Strobane				Toxaphene			
	Emulsion		Suspension		Emulsion		Suspension	
	No. 1	No. 2	No. 3	No. 4	No. 1	No. 2	No. 3	No. 4
Before spraying	0	0	0	0	0	0	0	0
After first spray								
1	0.61	0.87	0.45	0.73	0.69	0.51	0.72	0.56
2	.52	.70	.53	.69	.67	.55	.66	.82
3	.36	.45	.44	.49	.50	.37	.60	.56
5	.24	.17	.17	.24	.23	.23	.30	.23
7	.07	.07	.16	.13	.19	.13	.28	.11
14	.13	0	.08	0	.07	.06	.25	0
21	0	.10	.10	0	.08	.07	.21	.03
After second spray								
1	0.47	0.46	0.57	0.45	0.47	0.57	0.89	0.54
2	.55	.55	.69	.66	.51	.59	.92	.70
3	.29	.45	.53	.52	.42	.40	.66	.48
5	.13	.22	.40	.38	.24	.25	.40	.22
7	.04	.08	.17	.16	.15	.16	.20	.15
14	0	.02	.03	.04	.09	.10	.14	.02
21	0	.04	.02	0	.06	.05	.04	0